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Evaluation of Mycorrhizal Fungi in Container Grown Red Pine Seedlings

by

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TABLE OF CONTENTS

ACKNOWLEDGMENTS 1

INTRODUCTION 3

OBJECTIVES 4

MATERIALS AND METHODS 4

RESULTS AND DISCUSSION 9

a. Harvest #1 10

b. Harvest #2 11

SUMMARY 12

BIBLIOGRAPHY 14

INTRODUCTION

Mycorrhiza, as defined by Frank (1885) means fungus root. Since the discovery of mycorrhizae basic and applied scientists have become increasingly interested in the ecology and physiology of this form of symbiosis. Most economically important woody plants are obligately mycorrhizal in nature. Ectomycorrhizae accounts for nearly 5% of the world's vascular plants including Pinaceae (Trappe, 1977). Ecto-mycorrhizal pine seedlings are more tolerant of harsh growing conditions than are asymbiotic pine seedlings (Marks & Kozlowski, 1973). The fungus component utilizes excess plant metabolites while effectively increasing the water and nutrient absorbing capacity of the tree root system. The mycorrhizal fungus is also capable of deterring root pathogens, protecting seedlings from root toxins, and stimulate seedling biomass by production of growth regulators (Trappe, 1977). Thus, seemingly both the fungus and host plant mutually benefit.

Current research data underline the importance of mycorrhizal development on nursery grown pine seedlings.

Studies conducted at the United States Forest Service tree seedling nurseries (Eveleth, MN and Toumey, MI) during the summer of 1975 (Anderson, et al.) showed poor mycorrhizal development on one (1) through four (4) year old red pine (Pinus resinosa) seedlings. Maximum ectomycorrhizal (cited as ECM throughout the remainder of the text) development was observed to be approximately 15% of the total number of feeder roots on four-year-old seedlings. Poor ECM development could only be attributed to a cumulative effect of a number of variables identified in modern tree seedling nursery management.

This evaluation was initiated to study selected ECM fungi in producing desirable containerized grown red pine seedlings. Containerized grown seedlings are attracting wider usage throughout the United States as a seedling source in reclamation and reforestation. Moreover, this is an efficient and manageable system for comparative evaluation of various fungi on mycorrhizal development through time.

OBJECTIVES

1. Determine host (Pinus resinosa) growth-promoting ability of Boletus spectabilis Peck (preferred name: Fuscoboletinus spectabilis (Peck) Pomerleau & Smith), Thelephora terrestris Ehrh. ex. Fr. and Pisolithus tinctorius (Pers.) Coker & Couch.

MATERIALS AND METHODS

ECM Fungi Evaluated

1. T. terrestris: Wisconsin isolate
2. P. tinctorius: Wisconsin isolate
3. P. tinctorius: Georgia isolate
4. B. spectabilis: Minnesota isolate

Inoculum Preparation

Selected ECM fungi were grown on a modified Melin-Norkrans medium* as follows:

Sucrose or glucose	10 g
$(NH_4)_2HPO_4$	0.25 g
KH_2PO_4	0.50 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$CaCl_2$ (stock solution**)	5.0 ml/l

NaCl (stock solution)	1.0 ml/l
$FeCl_3$ (stock solution)	10 drops/l
Thiamine HCl (stock solution)	1.0 ml/l
Malt extract	3.0 g
Bacto agar	15 g
DH_2O	1000 ml

*Routinely adjusted to pH 5.5-5.7 by adding 1.5 ml 8.5% lactic acid to cooling agar.

**Formula for stock solutions used in growing mycorrhizal fungi:

$CaCl_2$: 10.00 grams powder/1 DH_2O

NaCl: 25.00 grams crystals/1 DH_2O

$FeCl_3$: 1.62 grams powder/100 ml DH_2O

Thiamine HCl: 1.00 gram of powder/1 DH_2O

Ectomycorrhizal fungi were allowed to grow across the agar substrate (3-4 wk required) then aseptically transferred into four (4) liter glass jars containing a 4:4:2:1 mixture of peat moss:vermiculite:perlite:topsoil. Fifteen hundred ml of Melin-Norkrans nutrient broth was aseptically added to the mixture then both were autoclaved. Each jar lid was modified with an air lock designed to prevent contamination by airborne spores.

Melin-Norkrans nutrient broth*

Glucose, sucrose	4.0 g
$(NHu)_2\ HPO_4$	0.25 g
KH_2PO_4	0.50 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2$ (stock solution)	5.0 ml/l
$NaCl_2$ (stock solution)	1.0 ml/l

Sequestrene (stock solution)	1.0 ml/l
Thiamine HCl (stock solution)	1.0 ml/l
Distilled water (DH_2O)	990 ml

*Routinely adjusted pH using procedure previously described.

The inoculated mixture was incubated at room temperature for four (4) to five (5) months to allow the ECM fungi to completely ramify the substrate. The ECM inoculum is the mixture colonized by the fungi.

Seed Preparation

Northern ecotype red pine seeds obtained from Potlatch Corp. were surface sterilized in 15% reagent grade H_2O_2 for five (5) minutes with constant swirling using a magnetic plate and stirring bar. Seeds were subsequently taken through two five (5) minute rinses in sterile distilled water prior to use.

Styroblock Preparation and Planting

Styroblocks Type 8 (obtained from Silva Seed Co., Roy WA) were used in this evaluation. Each block consists of four sections containing 20, 8 cu in cavities per section for a total of 80 cavities per block.

Each cavity was sprayed with 70% ETOH, plugged with a sterile Kimwipe to prevent loss of the inoculum and potting mixture through the drain hole (but allowing free passage of water). Each cavity was then filled with 6.0 cu in of the 4:4:2:1 mix minus the nutrient broth. The inoculum containing the ECM fungi was layered (1.6 cu in) on top of the potting mixture. Two surface sterilized red pine seeds were placed directly on the inoculum layer. A thin layer of potting mixture was used to "cap" the cavity. Sterilized granite chicken grit was used as the final topmost layer and its purpose was to stabilize the cavity contents (Figure 1).

Treatments

The statistical design used in this evaluation was a complete randomized model; treatments were assigned to a quarter block using double hat determination. Each treatment was replicated.

Specific treatments:

1. Control: (without ECM) no fertilizer
2. Control: (without ECM) 15-15-15 NPK
3. B. spectabilis: no fertilizer
4. B. spectabilis: 15-15-15 NPK*
5. T. terrestris: no fertilizer
6. T. terrestris: 15-15-15 NPK
7. P. tinctorius: Georgia #138, no fertilizer
8. P. tinctorius: Georgia #138, 15-15-15 NPK
9. P. tinctorius: Wisconsin-Burbee, no fertilizer
10. P. tinctorius: Wisconsin-Burbee, 15-15-15 NPK

(*Fertilizer applied at 50 ppm, 10 ml weekly per cell)

Seedlings were harvested at five (5) and ten (10) months after planting. The following data were recorded for each seedling.

1. Shoot length
2. Root length
3. Shoot dry weight
4. Root dry weight
5. Per cent mycorrhizal formation
6. Random sampling for ECM formation using cryostat sectioning and subsequent examination by Nomarsky differential interference phase microscopy.

Shoot length was measured to the nearest tenth millimeter from the stem base to the tip of the terminal bud. Root length was measured to the nearest tenth millimeter from the root collar to the tip of the longest rootlet.

Per cent feeder roots exhibiting mycorrhizal formation were determined by a total count of non-mycorrhizal and mycorrhizal rootlets for each seedling.

After per cent mycorrhiza was determined shoot and root dry weights were obtained by first placing shoots and roots in glycine packets, then in a convection drying oven held at 65 degrees C for 3 days. The glycine packets were then removed from the oven, and immediately placed in a chamber containing $CaCl_2$ for 15 minutes to allow the tissue to cool to room temperature at which time dry weights were taken to the nearest one thousandth of a gram.

Random samples of forked rootlets were sectioned by hand and cryostat to substantiate mycorrhizae.

RESULTS AND DISCUSSION (GENERAL)

Cavities in which multiple emergence occurred required thinning to one seedling per cavity. All but the most robust seedling in each cell was eliminated by cutting off the shoots at the surface of the growth mixture. Overseeding requires thinning, but represents a significant time savings.

Initial seedling emergence was 96%. Emergence failure was attributed to causes other than damping off.

Mycorrhizal formation in the controls likely occurred from airborne spores dispersed from ECM fungi landing in the cavities. Seedling growth and mycorrhizal development in those treatments was erratic enough to suggest the need for a planned inoculation program using selected ECM fungi.

Results and Discussion: five month harvest (H-1)

Computer generated data on H-1 seedlings showed T. terrestris with and without fertilizer forming superior mycorrhizae over P. tinctorius and B. spectabilis (Table H-1). Other parameters (Table H-1) were less significant at first harvest. However, red pine seedlings grown with T. terrestris and P. tinctorius with fertilizer and alone consistently produced superior seedlings over similar treatments with B. spectabilis and the controls.

Summary:

1. Thelephora terrestris used as inoculum produced superior mycorrhizae at five (5) months over P. tinctorius and B. spectabilis.
2. Data generated from seedlings harvested at five (5) months should be considered preliminary data, only suggestive of later developmental trends in biomass production and mycorrhizal formation.
3. Subsequent research funded by University of Minnesota, Institute of Agriculture, will determine survival of five (5) month container grown mycorrhizal seedlings in plots.

Results and Discussion: (H-2)

Root length (RL) (Table H-2) of container grown red pine seedlings appeared to be influenced to some extent by depth of cavity. However, data analysis showed that seedlings inoculated with T. terrestris alone were significantly longer than from other treatments. Root length data when compared to root dry weight data suggest a poor correlation between RL and total root biomass production on a dry weight basis.

Shoot length (SL) (Table H-2) of seedlings grown with B. spectabilis and fertilizer were significantly longer than the controls and red pine with B. spectabilis alone (Figure 2,3). Red pine seedlings grown with T. terrestris significantly increased SL over the controls (Figure 3) and seedlings grown with T. terrestris without fertilizer (Figure 4). Red pine seedlings grown with P. tinctorius and fertilizer significantly increased SL over the controls (Figure 3) and red pine grown with P. tinctorius alone (Figure 5). No significant differences occurred between seedlings grown with either the Wisconsin or Georgia isolate of P. tinctorius. Fertilized red pine seedlings grown with B. spectabilis, T. terrestris, and P. tinctorius showed no significant differences in SL (Figure 6).

Root dry weight (RDW) (Table H-2) and shoot dry weight (SDW) (Table H-2-4) of red pine seedlings grown with B. spectabilis and fertilizer were significantly greater than the controls and red pine with B. spectabilis alone. Red pine seedlings grown with T. terrestris and fertilizer significantly increased RDW and SDW over the controls and seedlings grown with P. tinctorius alone. Fertilized red pine seedlings grown with B. spectabilis, T. terrestris and P. tinctorius showed no significant differences in RDW and SDW.

Red pine seedlings grown in the presence of T. terrestris with fertilizer produced seedlings with the greatest stem diameter (Table H-2). However, no significant differences were detected among or between treatments.

Ectomycorrhizal formation on red pine seedlings grown with B. spectabilis was quite variable among replications. Boletus spectabilis does not normally occur in association with red pine in natural habitats. This study has shown that mycorrhizae can be established between "non-specific" hosts such as red pine and B. spectabilis under controlled conditions. Results such as these provide some insight on the ecology of these organisms particularly in reference to off-site plantings in reforestation.

Ectomycorrhizal development on red pine grown with T. terrestris and fertilizer was significantly higher than other treatments (Table H-2).

Ectomycorrhizal development on red pine grown with P. tinctorius and fertilizer was less than that grown with P. tinctorius alone. However, the difference was not significant except in the case of P. tinctorius, Georgia isolate. Georgia and Wisconsin isolates of P. tinctorius formed ECM of comparable quantity.

Root-shoot ratios based on a comparison of dry weight analysis was close to a one to one ratio at ten months; a ratio considered desirable in seedling production.

Summary:

1. Seedling growth and mycorrhizal development were enhanced in treatments receiving ECM fungi in combination with moderate fertilizer applications (15-15-15).
2. The method used for inoculating seedlings in this evaluation appears to be suitable for container-grown plants.

3. Plants grown in the presence of T. terrestris and fertilizer appeared to be consistently superior to plants with other treatments.

BIBLIOGRAPHY

1. Anderson, R. L., S. V. Krupa, P. R. Laidly and E. L. Stewart. 1976. Evaluation of ectomycorrhizae on red pine seedlings at Toumey and Eveleth National Forest nurseries in the lake states. Forest Insect and Disease Management Evaluation Report 5-14-76.

2. Frank, A. B. 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unteriridsche Pilze. Dtsch. Bot. Ges. Ber. 3:128-145.

3. Marks, G. C. and T. T. Kozlowski (ed.) 1973. Ectomycorrhizae - their ecology and physiology. Academic Press, New York and Canada. 444 pp.

4. Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopathol. 15:203-222.

Table H-1. Container grown red pine seedlings inoculated with selected mycorrhizal fungi.

Parameter	Fertilizer treatment	Control	Mycorrhizal Treatment[1] B. spectabilis	T. terrestris	P. tinctorius
Root length (cm)	0	15.28 abc*	14.66 a	18.88 f	18.50 f
	15-15-15		17.52 def	17.44 cdef	18.08 f
Shoot length (cm)	0	7.22 ab	14.44 fg	13.50 efg	14.33 fg
	15-15-15		14.41 fg	16.02 g	15.38 g
Root dry weight (g)	0	0.059 ab	0.148 e	0.135 d	0.158 e
	15-15-15		0.144 e	0.129 d	0.139 de
Shoot dry weight (g)	0	0.070 ab	0.242 efg	0.266 efgh	0.278 fgh
	15-15-15		0.240 defg	0.342 h	0.371 h
Stem diameter (mm)	0	0.75 c	0.99 f	1.10 gh	1.11 gh
	15-15-15		0.93 c	1.12 gh	1.14 gh
Per cent mycorrhizal rootlets	0	0.622 a	4.69 ab	43.78 d	7.16 ab
	15-15-15		4.84 ab	23.29 c	10.67 b

*Values not sharing the same letter are significantly different (Tukey w-procedure at the 5% level).

[1]Control = mycorrhizal fungi not added.

Table H-2. Container grown red pine seedlings inoculated with selected mycorrhizal fungi.

Parameter	Fertilizer treatment	Control	Mycorrhizal Treatment[1]			
			B. spectabilis	*T. terrestris*	*P. tinctorius* Wisconsin isolate	*P. tinctorius* Georgia isolate
Root length (cm)	0	15.37 abcd*	18.09 fgh	19.25 h	16.75 defg	15.65 abcd
	15-15-15	15.77 abcde	16.35 bcdef	15.97 bcde	16.55 cdefg	15.30 abcde
Shoot length (cm)	0	8.26 ab	9.42 abcd	12.02 d	11.22 cd	11.45 cd
	15-15-15	10.55 bcd	16.625 e	16.86 e	15.27 e	15.32 e
Root dry weight (g)	0	0.077ab	0.111ab	0.196 cdefg	0.197 defg	0.214 fgh
	15-15-15	0.204 efg	0.425 d	0.413 j	0.393 ij	0.424 j
Shoot dry weight (g)	0	0.0817abc	0.083abc	0.157 bcd	0.167 bcd	0.197 d
	15-15-15	0.148 bcd	0.465 fg	0.395 efg	0.347 e	0.471 g
Stem diameter (mm)	0	0.74 a	0.97 a	1.04 a	1.05 a	1.05 a
	15-15-15	1.01 a	1.54 a	1.42 a	1.37 a	1.51 a
Per cent mycorrhizal rootlets	0	7.01 abc	5.75 ab	20.91 defg	31.64 fg	21.40 defh
	15-15-15	13.63 abcd	19.63 cdef	62.72 h	20.05 cdef	17.19 bcde

*Values not sharing the same letter are significantly different (Tukey w-procedure at the 5% level).

[1]Control = mycorrhizal fungi not added.

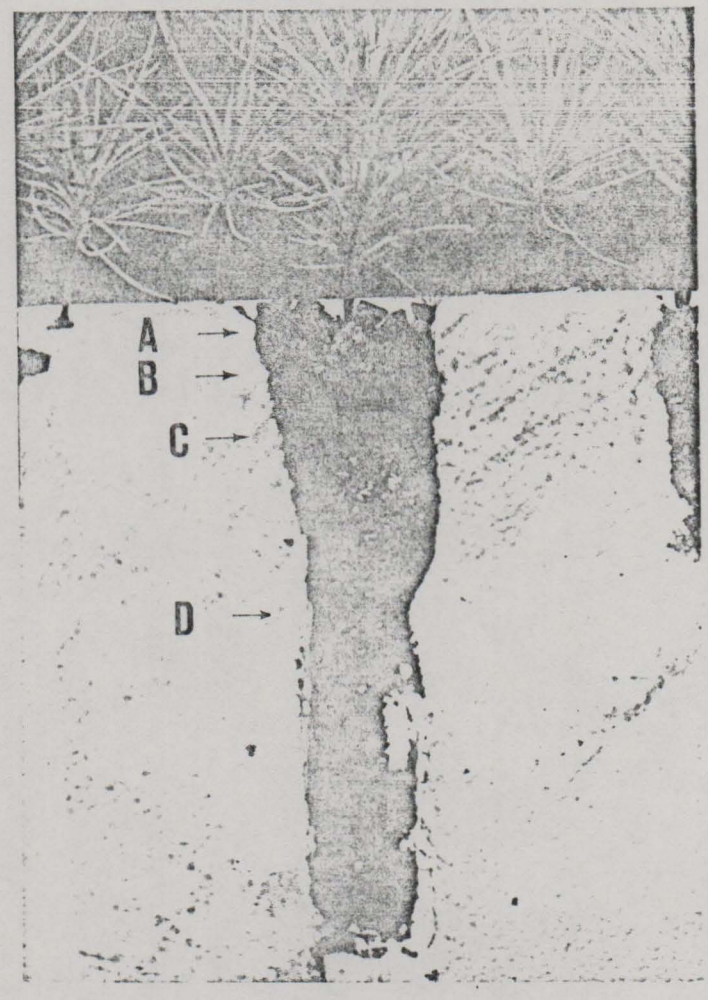


Figure 1. Section of styroblock showing layers of potting mix.

A. Granite chicken grit. B. Potting mixture.

C. Layer of ECM inoculum. D. Potting mixture.

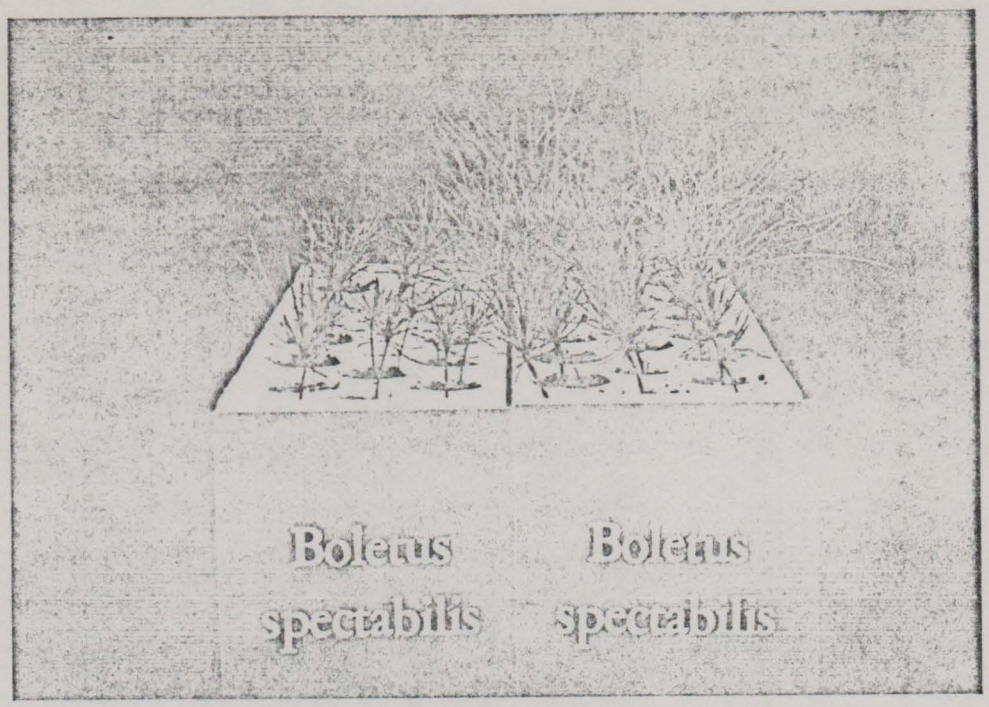


Figure 2. Container grown mycorrhizal seedlings with B. spectabilis and fertilizer (red flag) and alone.

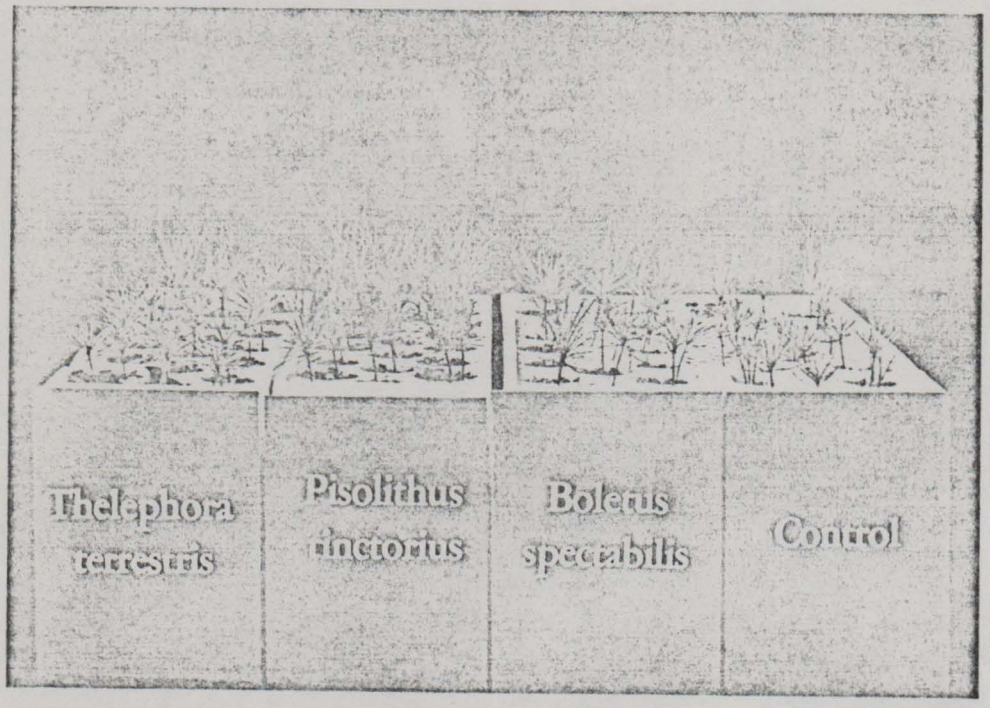


Figure 3. Comparison of container grown mycorrhizal seedlings with ECM fungi and control.

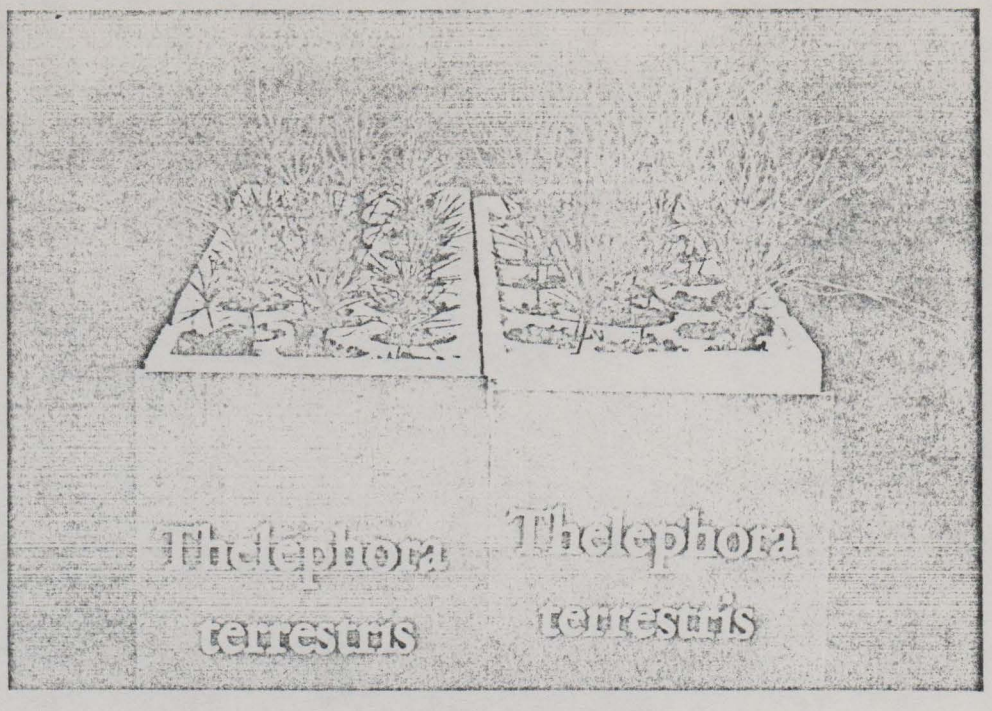


Figure 4. Container grown mycorrhizal seedlings with T. terrestris and fertilizer (red flag) and alone.

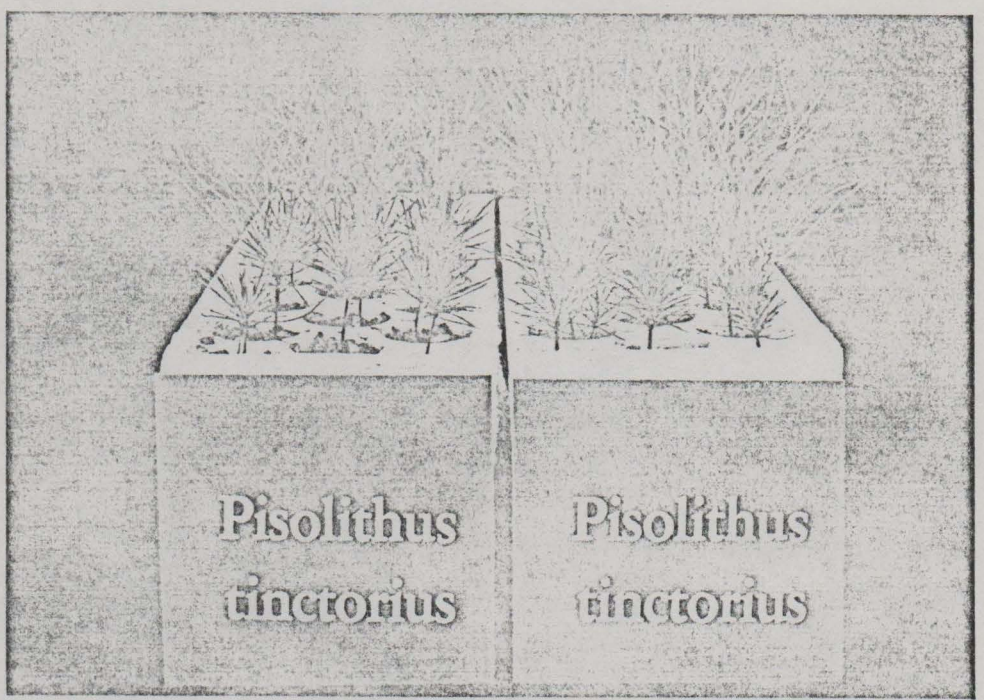


Figure 5. Container grown mycorrhizal seedlings with P. tinctorius and fertilizer (red flag) and alone.

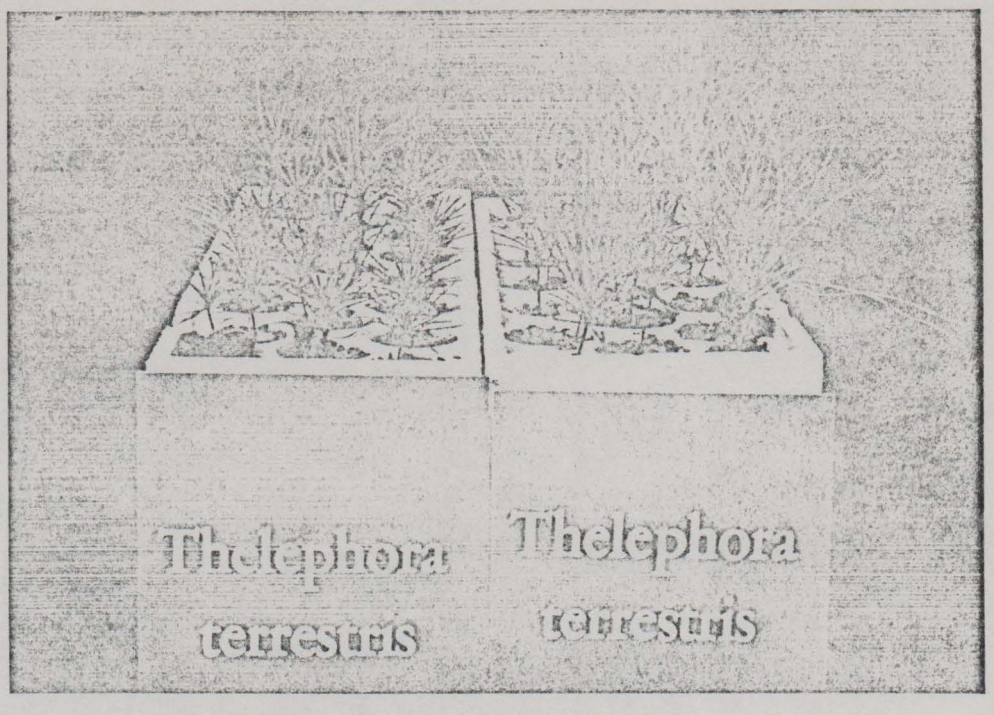


Figure 4. Container grown mycorrhizal seedlings with T. terrestris and fertilizer (red flag) and alone.

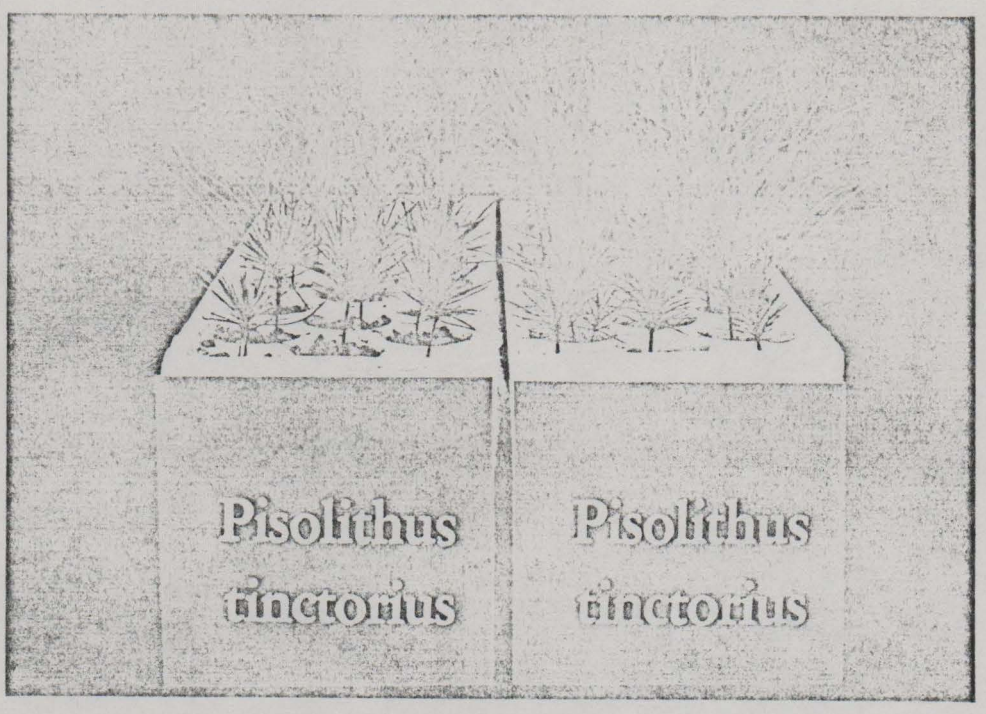


Figure 5. Container grown mycorrhizal seedlings with P. tinctorius and fertilizer (red flag) and alone.

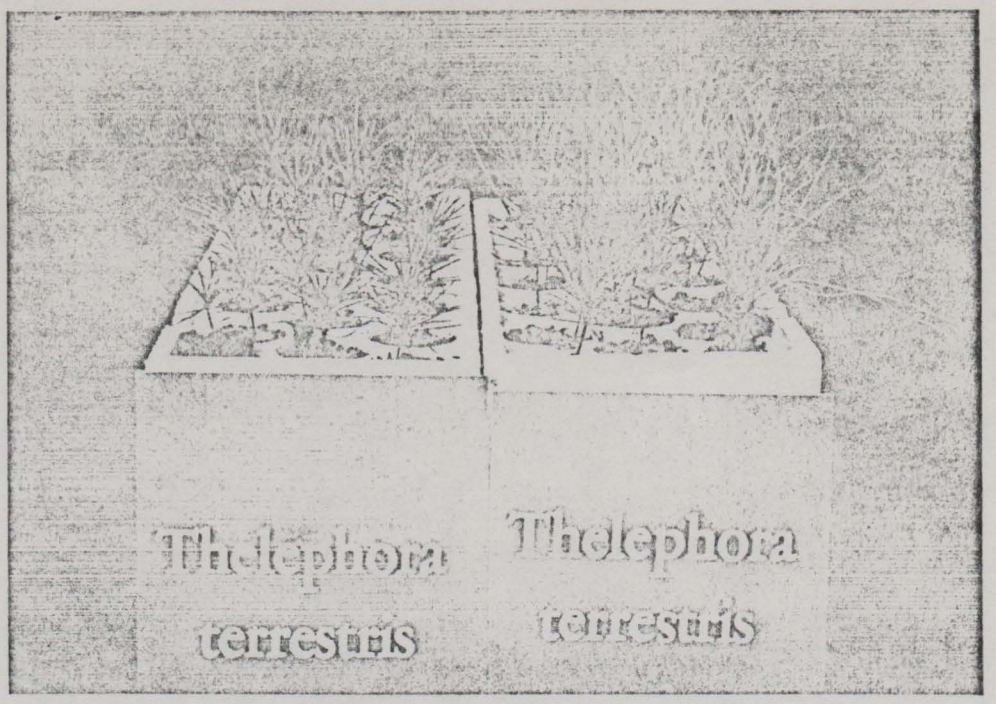


Figure 4. Container grown mycorrhizal seedlings with T. terrestris and fertilizer (red flag) and alone.

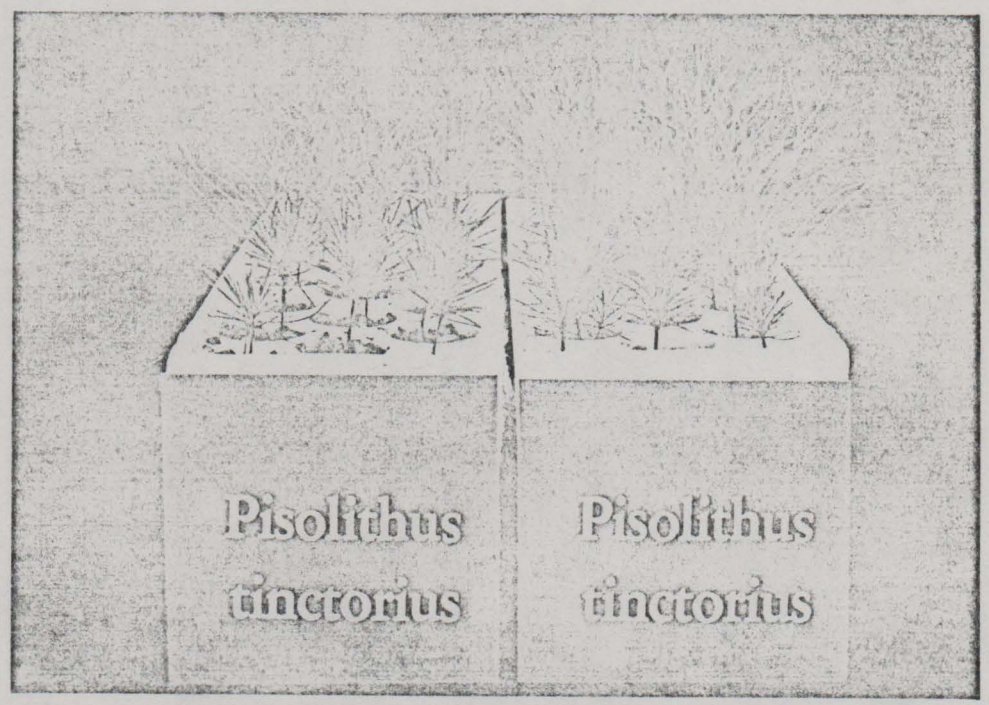


Figure 5. Container grown mycorrhizal seedlings with P. tinctorius and fertilizer (red flag) and alone.

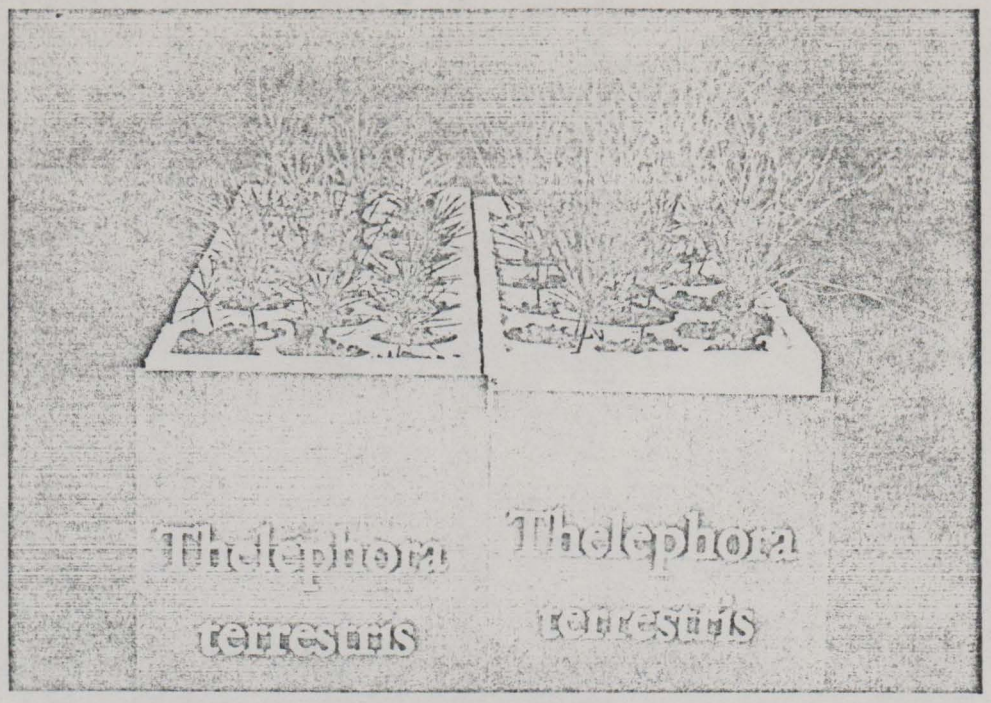
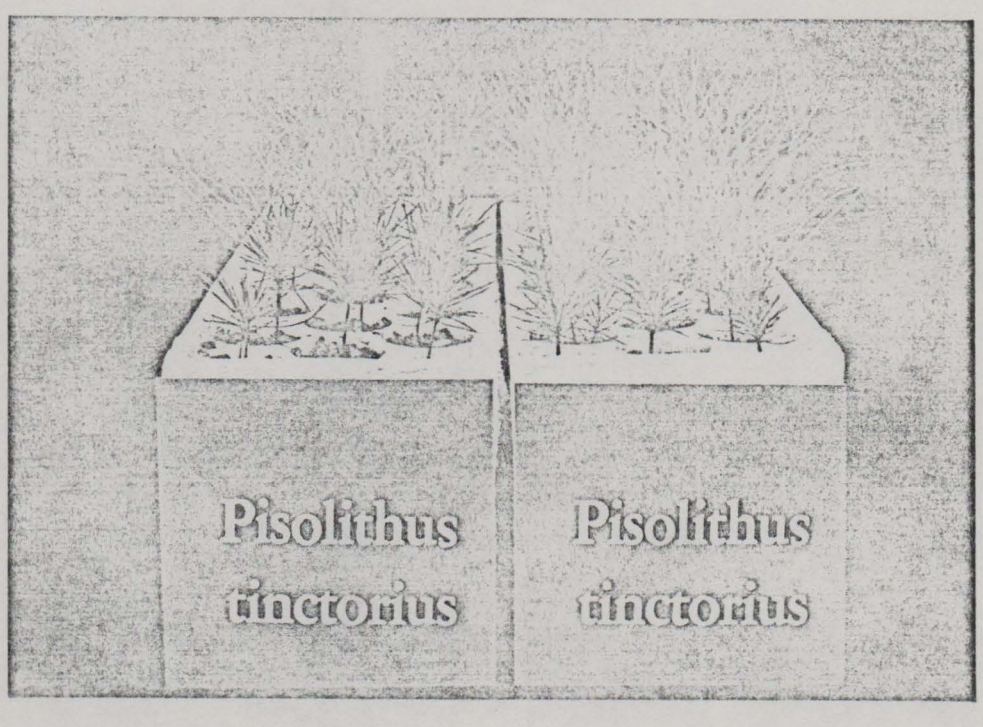


Figure 4. Container grown mycorrhizal seedlings with T. terrestris and fertilizer (red flag) and alone.



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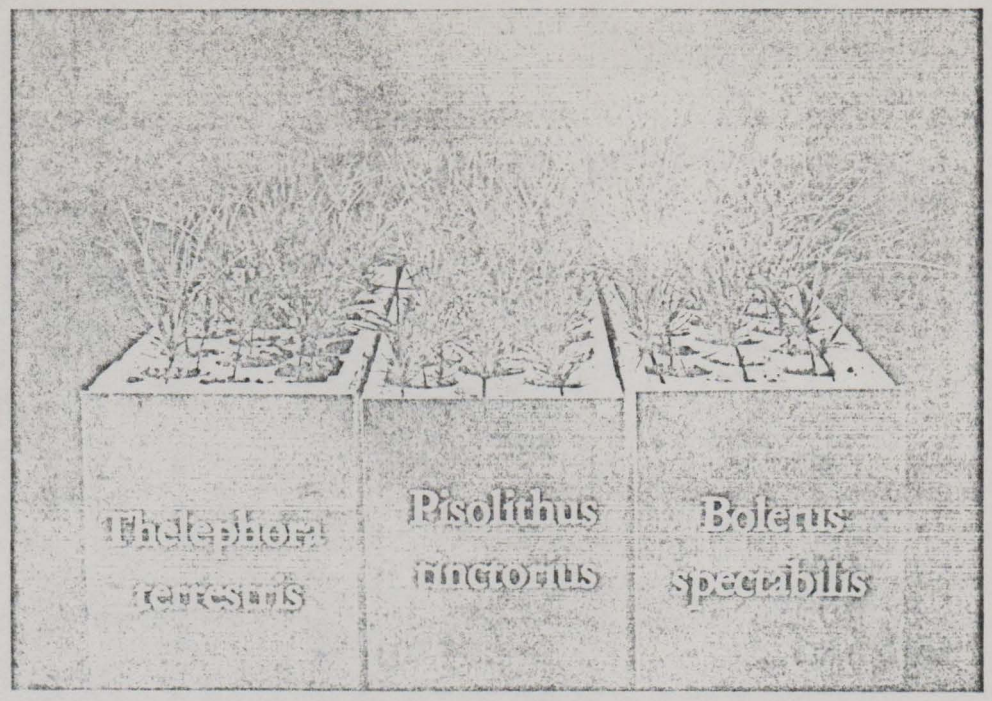

Figure 6. Container grown mycorrhizal seedlings with fertilizer (red flags), T. terrestris, P. tinctorius, and B. spectabilis.

Figure 7. Typical ECM of T. terrestris on red pine (approximately 10X).

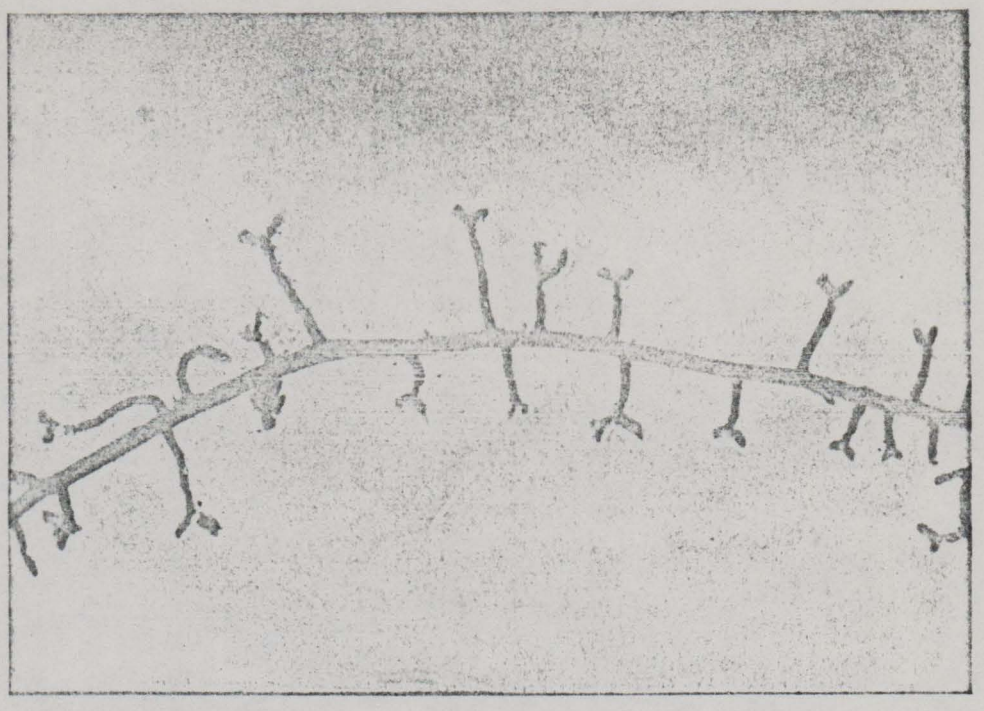

Figure 8. Typical ECM of P. tinctorius on red pine (approximately 4X)